# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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**Supplementary Material**

# A novel prion disease presenting with diarrhoea and autonomic neuropathy

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## Table of Contents

**Supplementary Case Material**

***Patient IV.A***

*History*

The patient presented with a profound autonomic syndrome, characterised by abdominal pain, faecal urgency and watery diarrhoea at the age of 33, erectile dysfunction at the age of 38, and urinary incontinence by the age of 42. Significant postural hypotension secondary to autonomic failure developed in his fourth decade. Distal numbness and paresthesiae, accompanied by distal weakness, was initially noted in his hands and legs in the third decade and gradually progressed. By the age of 58, due to sensory ataxia, he required a wheelchair outside the house but was able to walk indoors. He complained of sharp pains in the soles of his feet at this time. His wife noted that he had become 'twitchy' in his late fifties, and this was attributed to his neuropathy. Four years prior to his death he consented to brain donation and, in a questionnaire, described his own symptoms as slowness of movement, involuntary jerking movements, worsening of balance with frequent falls, occasional confusion, forgetfulness, memory loss, and depression. History taken from family members suggests a ten year period from the age of 55 during which he was intermittently confused, increasingly fidgety, had a notable personality change, and suffered from insomnia. In addition he described two generalised seizures, at the ages of 56 and 62.

He required several long hospital admissions for severe unremitting diarrhoea and was treated initially with pancreatic enzyme supplementation and subcutaneous octreotide, and later required parenteral nutrition via a Hickman line. An indwelling urinary catheter was used to manage the urinary incontinence. The postural hypotension was managed with nocturnal DDAVP nasal spray and a high salt diet. Neuropathic pain was treated with carbamazepine. He died of bronchopneumonia at the age of 66.

*Examination*

Significant postural hypotension was consistently noted, with a fall in blood pressure from 135/95 mmHg lying to 95/65 mmHg on standing. General examination was unremarkable. Neurological examination at the age of 58 revealed some involuntary lip smacking and sniffing movements. Cranial nerve examination was normal apart from an absent pupillary reaction to light. There was marked distal wasting of the limbs, accompanied by 4/5 reduction in power in the distal muscles of both upper and lower limbs. All tendon reflexes were absent, and plantar responses were flexor. There were no obvious cerebellar features. All sensory modalities were intact in the upper limbs but sensation to light touch and pinprick was absent below the knee; vibration sense was absent to the ankle. Joint position sense was reduced to the ankle. There was a positive Romberg's sign and a high stepping gait.

*Investigations*

Blood tests: Urea and electrolytes, liver function tests, thyroid function tests, glucose, B12, folate, full blood count, ESR, CRP, treponemal serology, autoantibody screen were normal or negative. Protein electrophoresis demonstrated an IgM paraproteinaemia at 4.2g/dl (in the presence of a normal bone marrow biopsy) – the overall opinion of the treating physicians was that this finding was incidental to the neuropathy. An echocardiogram was normal. A serum amyloid protein (SAP) scan revealed only equivocal deposits in both kidneys. Biopsies of the stomach, duodenum, large bowel and rectum, and a renal biopsy had been reported as histologically normal without evidence of amyloid deposition, but were later destroyed**.** A sural nerve biopsy performed 9 years before death was reported to show severe loss of myelinated and unmyelinated axons with no significant regeneration. Amyloid accumulation

could not be detected using either Congo red staining or on ultrastructural examination. No widely spaced myelin was reported.

Neurophysiology studies demonstrated a severe axonal sensorimotor neuropathy with absent sural SNAPs, absent motor responses in distal muscles and attenuated CMAP amplitudes in proximal muscles with mildly reduced conduction velocity (supplementary table 1). EMG showed chronic partial denervation. Autonomic function testing revealed severe postural hypotension: the supine BP was 110/63 mmHg, falling to 55/15 mmHg after one minute of a 45º head tilt. Pressor responsiveness was preserved to isometric exercise, mental arithmetic and cutaneous cold. There was a markedly abnormal fall in the blood pressure during exercise but a minimal fall in BP to clonidine. Genetic testing for common transthyretin mutations and *SPECT1* were negative.

***Patient IV.D***

*History*

Patient presented at the age of 30 with watery diarrhoea which progressed to a frequency of 6 times a day within ten years. This was diagnosed by gastroenterology as "irritable bowel". Incomplete bladder emptying developed at the age of 39 necessitating intermittent self-catheterisation. At age 40 she developed postural dizziness and dry eyes. Distal numbness and paresthesiae of her feet was initially noticed in her fourth decade, and progressed gradually with significant neuropathic pain and weakness. She first developed paranoia, aggression and depressive symptoms during a hospital admission at age 52 and was treated with mirtazepine. Memory problems were apparent at age 52 and intermittent confusion from age 55. There is no history of seizures, myoclonus, or sleep disturbance.

She has been treated with carbamazepine, gabapentin and amitryptiline for the neuropathic pain. A PEG feeding tube was inserted at the age of 50 to help manage the gastrointestinal dysfunction. Postural hypotension was treated with fludrocortisone.

Aged 56 she became severely unwell with diarrhoea, vomiting, weight loss and confusion necessitating a prolonged hospital admission. Diarrhoea did not respond to antibiotic treatment, loperamide, opiates or dietary measures. Her weight was only controlled following parenteral nutrition via a Hickman line. She died aged 59 and a full autopsy was conducted.

*Examination*

Examination aged 49 revealed supine blood pressure was 129/75 mmHg falling to 100/68 mmHg on standing. There was a reduction in sensation of pinprick and temperature to the knees and vibration sense and joint position sense to the ankles. Romberg's test was positive. All reflexes were absent. Distal wasting and weakness of the toe extensors was present. There was no evidence of cerebellar dysfunction.

In 2003, a detailed neuropsychological assessment showed a verbal IQ of 80 and a performance IQ of 92, reflecting significant cognitive decline. Memory functions were severely affected with performance on the Recognition Memory Test for Words and Faces both at chance level. Performance on the Graded Difficulty Spelling Test was poor ($1^{st}$-$5^{th}$ percentile). Abnormalities were detected on several tests sensitive to frontal executive function. Visual perceptual and spatial abilities and object naming were intact. Three years later there was significant progression with a decline in general intellectual function (with verbal IQ 67, performance IQ 72), and most aspects of cognition were more impaired while

visuospatial function and praxis remained relatively intact. Sentence comprehension and non-word reading were poor in comparison to relatively intact synonym matching.

*Investigations*

Blood tests, including serum protein electrophoresis, were normal. An echocardiogram was normal. A SAP scan showed only equivocal deposits in the spleen. A rectal biopsy was histologically normal with no evidence of amyloid deposition but was not available for further study and no PrP immunohistology was done. MRI brain showed only a couple of tiny discrete white matter signal abnormalities and electroencephalograms (EEGs) showed no specific changes of prion disease. The neurophysiological studies showed a length-dependent sensorimotor axonal polyneuropathy with small fibre involvement. Over the 10 year period there was progressive deterioration of SNAP and CMAP amplitudes starting in the legs before affecting the upper extremities. There was a mild slowing of conduction velocity and proportionate slowing of the F-wave latencies which might have been the consequence of a drop out of the dropout of axons of the largest calibre. EMG showed chronic neurogenic changes in tibialis anterior (1998) and peroneus tertius (2009). (supplementary table 1) Thermal thresholds remained normal throughout whereas there was progressive deterioration in the legs.

Autonomic function testing demonstrated a fall in BP on head tilt in association with a blocked valsalva manoeuvre. There was evidence for both sympathetic and parasympathetic autonomic dysfunction. There was a slow pupillary reaction to light with redilatation lag.

***Patient IV.B***

*History*

The first symptom was post-prandial diarrhoea in the fourth decade. Stool frequency was 3-4 x /day, watery in nature. Impotence and postural hypotension developed in the fifth decade. Around this time, he developed urinary incontinence and required a suprapubic catheter insertion. During the same period he developed a severe sensorimotor neuropathy associated with neuropathic ulcer formation on his feet. In his late 50's, it was noted that he had deteriorating intellectual function with an inability to acquire new information and a fluctuating memory. He died of acute renal failure in his $7^{th}$ decade.

*Examination*

Examination at the age of 58 showed lying BP 167/109 mmHg, pulse 73; standing BP 69/45 mmHg pulse 90 at 1 min and 67/40 mmHg pulse 90 at 3 minutes. Cardiovascular, respiratory and abdominal examination was unremarkable. An Addenbrooke's Cognitive Examination score was 38/100. His language fluency, repetition, and comprehension were normal. There was a mild postural tremor of the upper limbs. There was no gait or limb ataxia. The postural reflexes were impaired. Cranial nerve examination was normal. Tone was normal. There was wasting and weakness distally. Tendon reflexes were intact in the upper limbs, but absent in the lower limbs. There was reduced vibration sense to the wrist and knee. Pinprick sensation was reduced in a glove and stocking distribution.

*Investigations*

Blood tests, including autoantibodies and protein electrophoresis, were normal.

A SAP scan was normal. A rectal biopsy did not show evidence of amyloid deposition but was not available for further study and PrP immunohistology was not performed. Neurophysiology studies confirmed small or absent sensory and motor responses in the lower limbs more than the upper limbs, consistent with a severe axonal sensorimotor neuropathy. A CT brain scan in the 5$^{th}$ decade was normal. An EEG demonstrated irregular slow activity compatible with an encephalopathy.

***Patients II.B, III.A, C, F***

A clinical syndrome of autonomic dysfunction characterised by diarrhoea, bladder dysfunction and postural hypotension and syncope in association with symptoms of a sensorimotor neuropathy was termed the 'family neuropathy' and was described to have affected family members II.B, III.A, C, F. The exact ages of onset could not be ascertained but were recounted by the family members as generally in the 4th/5th decades. The cause of death for III.A was unclear and a post-mortem was performed which defined aortic stenosis as cause of death and characterised the brain as 'grossly normal'.

***Patient III.E***

She is reported to have developed chronic diarrhoea in her early 30's. She later had problems with her memory and died at the age of 48. Also reported to have had thyroid problems and her death certificate states one of the causes of death as thyroid cyst rupture/thyrotoxicosis.

***Patient IV.F***

*History*

This 52 year old right handed woman had unexplained bouts of chronic diarrhoea with abdominal cramps and occasional faecal incontinence from the late fourth decade. She lost approximately two stone in weight despite well-preserved appetite. Following a colonic biopsy she was diagnosed with Crohn's disease and treated with steroids, although this diagnosis was thought to be somewhat equivocal. By her mid-fifties she had urinary incontinence, mild postural symptoms and peripheral neuropathy. She also complained of progressive deterioration in memory. This was initially evident as lapses in attention leading to mistakes in the course of her work as a teacher's assistant, and she took up a new job as a supermarket attendant. Subsequently, her recall of daily events and names declined, she became repetitive in conversation and frequently misplaced items around the house although she did not become lost in the local area. Her writing deteriorated and she made uncharacteristic spelling errors. Intermittently, and particularly at night-time, she would misperceive household objects as people; there was no history of auditory hallucinations or misperceptions. She experienced significant anxiety and depression and poor sleep, and expressed paranoid ideas from time to time. She separated from her husband, became fearful of leaving her house and moved to sheltered accommodation. She died following a fall and gastrointestinal haemorrhage. An autopsy was done.

Other past medical history included chronic normocytic anaemia at age 40, corneal ulcers and xerostomia from age 49, osteoporosis and a hip fracture following a fall at age 50, and hypothyroidism for which she was taking thyroxine replacement. Her other medications comprised citalopram, alendronic acid and calcium supplements.

*Examination findings*

On examination aged 55 she was thin and appeared anxious. MMSE score was 15/30, having been 21/30 a year earlier, losing points on orientation, recall and attentional tasks. Recall of current affairs was poor. On further bedside cognitive testing, speech was normal in structure and content, and comprehension of single words and sentences was intact. However, there were errors reading non-words and with spelling and arithmetic. There were perseverative responses on a verbal fluency task, and digit span was 5 forwards but only 3 reverse. There were errors of ideomotor praxis using both hands, however orofacial praxis was intact. On general neurological examination there was occasional stimulus-sensitive myoclonus of the fingers, and proprioception and vibration sense were reduced at the toes bilaterally; there were no abnormalities of distal power or muscles. Blood pressure lying was 120/70 mmHg, standing 90/60 mmHg. The systemic examination was otherwise unremarkable.

*Investigations*

Neuropsychological assessment demonstrated a verbal IQ of 76 and performance IQ of 84, reflecting a degree of intellectual decline. On focal cognitive tasks, performance on the Recognition Memory Test was at chance for verbal material but between the 10-25$^{th}$ percentile for faces, with impaired executive dysfunction on a simple Stroop test, impaired cognitive speed and attention. As was the Patient for IV.D, naming (Graded Naming Test) and visual perceptual functions were normal. Two years later the profile was similar but had progressed. Memory and executive functions were profoundly impaired, and speed of processing was slow, but visual perceptual and visual spatial functions and praxis were relatively spared. Again, as with IV.D, sentence comprehension and non-word reading were poor in comparison to relatively intact synonym matching.

Blood screens revealed mild normocytic anaemia. Inflammatory and paraneoplastic screens were negative.

An EEG showed preserved alpha rhythm with slow transients over the fronto-temporal regions bilaterally, more prominent on the left and with drowsiness, but no frank epileptiform features. Neurophysiological studies showed a length-dependent sensorimotor axonal polyneuropathy with small fibre involvement and length-dependent chronic neurogenic changes on EMG. Analysis of the CSF revealed 1 white cell, raised total protein (1.9 g/L), negative oligoclonal bands, positive 14-3-3 and raised S100 (2.17; normal <0.55), total tau (>1200) and amyloid beta 1-42 (1078). MR neuroimaging showed no specific features or volume loss.

***Patient V.B***

This patient developed diarrhoea aged 32, with nocturnal diarrhoea and fluctuating weight. Aged 39 she developed urinary hesitancy, retention was diagnosed and she began intermittent self-catheterization. She also developed shooting pains in the feet without signs of peripheral neuropathy. There was occasional postural hypotension, but no frank autonomic failure when tested in the laboratory. Examination findings including detailed neuropsychological assessment showed no clear abnormalities aside from unsteadiness during the Romberg's test, and variable postural hypotension. Investigations showed a positive hydrogen breath test. Subsequently her diarrhoea responded to treatment of bacterial overgrowth with tetracycline. SAP scan was negative. Neurophysiological studies showed evidence for a small fibre sensory and autonomic polyneuropathy affecting the legs. There were elevated thermal thresholds and absent sympathetic skin response in the feet, but normal results in the hands. Injection (100 μg) or iontophoresis

(15 mC) of histamine on two occasions did not eleict any itch sensation and produced a reduced flare response as measured with Laser-Doppler imaging. There was a normal cutaneous silent period (the afferent arch of this reflex is mediated by A delta fibres) in lower and upper extremities. Large fibre nerve conduction studies and EMG of TA was normal.

***Patient V.G***

This patient has been deliberately omitted to preserve anonymity and because comorbidies make her clinical features hard to interpret.

## Supplementary Methods

### *Patients and tissues*

The brain of the index patient (IV.A) was donated to the Queen Square Brain Bank for Neurological Disorders, UCL Institute of Neurology, London according to ethically approved protocols. The brain and multiple peripheral tissues from two further patients (IV.D, IV.F) were donated to the MRC Prion Unit. Numerous large and small bowel biopsies (patient V.B) were donated to the MRC Prion Unit. Patients were reviewed at the National Hospital for Neurology and Neurosurgery, or visited at home by staff of the National Prion Clinic, UCLH NHS Trust. Consent for genetic testing was obtained from living relatives. Clinical information relating to deceased subjects was acquired from the medical records, and by regular clinical assessment in the living family members. Storage and analysis of human tissue samples and transmission studies to mice were performed with consent from relatives and with approval from the Local Research Ethics Committee of the UCL Institute of Neurology/National Hospital for Neurology and Neurosurgery and complied with the code of practice specified in the Human Tissue Authority licence held by UCL Institute of Neurology. Patients with HSAN were recruited at the National Hospital Queen Square and archived in the Neurogenetics Unit, National Hospital.

### *Pathology*

*Neuropathology*

After fixation in 10% buffered formalin the brains were sliced in the coronal plane, tissue blocks were selected for histological examination and processed into paraffin wax using standard protocols including formic acid pre-treatment. Seven micron thick tissue sections were stained with routine methods including haematoxylin and eosin, luxol fast blue, periodic

acid-Schiff, Congo red and thioflavin S. Immunohistochemistry was performed using a standard avidin-biotin protocol using antibodies raised against: prion protein KG9 (gift from CJD Surveillance Unit, Edinburgh), UK; 3F4 (Dako, Ely, UK); ICSM35 (D-Gen, London, UK) and C-terminal antibody (Pri-917), amyloid P component (Novacastra, Newcastle, UK), glial fibrillary acidic protein (GFAP, Dako), tau (AT8, Autogen Bioclear, Calne, UK), tau-3R, tau-4R (gift from Dr R de Silva), amyloid-β (Dako), neurofilament cocktail (MP Biomedicals Inc., Aurora, Ohio, US), TDP-43 (Abnova, Taipei, Taiwan), CD68 (Dako), CR3/43 (Dako) and α-synuclein (Novocastra). The density of both tau and prion protein immunoreactive structures was assessed using a semi-quantitative scale: 0 = absent, + = mild, ++ = moderate, +++ = frequent. Negative controls are routinely used in all staining protocols.

*Sural nerve biopsy*

Five micron thick tissue sections from a formic acid pre-treated, paraffin embedded sural nerve biopsy performed on IV.A 9 years before death were examined following immunohistochemical staining for prion protein as above.

*Gastrointestinal biopsies*

Paraffin sections of duodenal biopsies performed in 2002 and 2003 from IV.F were examined following staining with Congo red, thioflavin S and immunohistochemical staining for prion protein as above.

*Transmission electron microscopy*

Formalin fixed samples from the temporal cortex were treated with formic acid followed by fixation in 3% glutaraldehyde buffered with 0.05M sodium cacodylate, pH7.4 and post-fixation in 1% osmium tetroxide. Tissue blocks were processed into resin using standard

protocols. Resin sections were stained with Toluidine blue and suitable regions were selected for electron microscopy. Ultra-thin sections were stained with lead citrate and examined in a Jeol 100-CXII electron microscope. Images were recorded on a 4 megapixel SIS Megaview digital camera.

***Molecular Genetics***

The entire open reading frame of *PRNP* was sequenced from genomic DNA using standard techniques[1]. Y163X mutation was confirmed using a Bfa-I restriction digestion. For phase analysis by allele specific PCR, primers were designed to specifically amplify either methionine or valine codon 129 related alleles. The mutation was detected by *PRNP* sequencing. Bfa-I restriction endonuclease digestion confirmed this mutation by cleavage of the wild-type amplicon of 1015bp at a single site to reveal products of 435bp and 580bp. Allele specific PCR amplification and sequencing of DNA from patients with the Y163X mutation revealed that the termination mutation at codon 163 is linked to valine at codon 129. Four patients were heterozygous at polymorphic *PRNP* codon 129 (IV.A, IV.D, IV.F, V.G) and one was homozygous for valine (V.B).

***Biochemistry***

All procedures were carried out in a microbiological containment level 3 laboratory with strict adherence to safety protocols. 10 % (w/v) frontal cortex homogenates were prepared in Dulbecco's phosphate buffered saline lacking $Ca^{2+}$ or $Mg^{2+}$ ions (D-PBS) by serial passage through needles of decreasing diameter or using tissue grinders. 10% (w/v) brain homogenates derived from transgenic mice expressing human PrP or wild-type mice were prepared in D-PBS and homogenized using Precellys-24 Ribolyser (Bertin Technologies) at 6500 rpm for 45s. Processed homogenates were separated from the beads using a 21G needle.

Homogenates were analysed with or without proteinase K (PK) digestion by electrophoresis and immunoblotting. Aliquots of 10 % (w/v) homogenate (typically 20 μl) were removed and PK added from a 1 mg/ml stock solution (prepared in water) to give a final concentration in the sample of 50 or 100 μg/ml. Following incubation at 37 °C for 1 h, samples were centrifuged at 16, 100 g for 1 min before termination of the digestion by the addition of an equal volume of 2 x SDS sample buffer (125 mM tris-HCl, pH 6.8 containing 20% (v/v) glycerol, 4% (w/v) sodium dodecyl sulphate, 4% (v/v) 2-mercaptoethanol, 0.02% (w/v) bromophenol blue and 8 mM 4-(2-aminoethyl)-benzene sulfonyl fluoride) and immediate transfer to a 100 °C heating block for 10 min. Non-PK digested samples were processed similarly with an equal volume of 2 x SDS sample buffer. Samples were centrifuged at 16, 100 g for 1 min prior to electrophoresis in 16% tris-glycine gels (Invitrogen). Gels were electroblotted onto PVDF membrane (Immobilon-P; Millipore) and subsequently blocked in PBS containing 0.05% v/v Tween-20 (PBST) and 5% (w/v) non-fat milk powder for 1 h. After washing with PBST blots were probed with anti-PrP monoclonal antibodies 3F4[2], or ICSM18 or ICSM35 (D-Gen Ltd, London) at 0.2 μg/ml final concentration in PBST in conjunction with anti-mouse IgG-alkaline phosphatase conjugated secondary antibody (Sigma; Cat No A2179) and chemiluminescent substrate CDP-Star (Tropix Inc, Bedford, MA, USA) and visualized on Biomax MR film (Kodak)[1]. Sodium phosphotungstic acid precipitation of disease-related prion protein from tissue homogenate was performed as described previously[1].

***Transmission studies***

All procedures were carried out in a microbiological containment level 3 animal facility with strict adherence to safety protocols. Care of mice was according to institutional and ARRIVE guidelines. Transgenic mice homozygous for a human PrP 129V transgene array and murine PrP null alleles (*Prnp*$^{o/o}$) designated Tg(HuPrP129V$^{+/+}$ *Prnp*$^{o/o}$)-152 mice (129VV Tg152 mice) or homozygous for a human PrP 129M transgene array and murine PrP null alleles (*Prnp*$^{o/o}$) designated Tg(HuPrP129M$^{+/+}$ *Prnp*$^{o/o}$)-35 mice (129MM Tg35 mice), and their use for transmission studies of a wide range of human and animal prion diseases, have been described previously[3-10]. Inbred wild-type FVB/NHsd mice were supplied by Harlan UK Ltd. Brain (frontal cortex) from IV.A was prepared as 1 % (w/v) homogenate in sterile D-PBS by serial passage through needles of decreasing diameter and 30 μl inoculated intracerebrally into groups of 8 129VV Tg152 mice and 129MM Tg35 mice and 8 FVB/N mice as described elsewhere[5-7]. Subsequently mice were examined daily and were killed if exhibiting signs of distress or once a diagnosis of clinical prion disease was established. Brains from inoculated mice were analysed by immunoblotting and neuropathological examination.

## Table S1

## Clinical features

| Patients | Estimated age at onset | Age at death | Gender | Diarrhoea | Bladder Symptoms | Neuropathy | Cognitive decline | Postural hypotension | Impotence |
|---|---|---|---|---|---|---|---|---|---|
| **II.B** | na | 70 | F | + | + | + | na | + | na |
| **III.A** | na | 68 | F | + | + | + | na | + | na |
| **III.C** | na | 42 | M | + | + | + | na | + | na |
| **III.E** | Early 30's | 48 | F | ++* | na | na | + | na | na |
| **III.F** | na | 55 | F | + | + | + | na | + | na |
| **IV.A** | 33 | 66 | M | ++* | ++ | ++ | + | ++ | + |
| **IV.B** | Early 30's | 66 | M | ++* | ++ | ++ | + | + | + |
| **IV.D** | 30 | 59 | F | ++* | + | ++ | + | + | na |
| **IV.F** | Late 30's | 55 | F | ++* | + | + | + | + | na |
| **V.B** | Early 30's | Alive | F | + | ++* | + | na | na | na |

Summary of clinical features as obtained from patient interviews, medical records and death certificates. '+' denotes record of a feature being present and '++' denotes a severe feature. '*' denotes initial presenting symptom. Missing, unconfirmed or not applicable data is represented by 'na'. [a]Death recorded as due to thyroid cyst rupture/thyrotoxicosis. [b]Death recorded as due to aortic stenosis. Note: patient V.G has been deliberately omitted from this table as comorbidies make her clinical features hard to interpret.

## Table S2

## Electrophysiological studies

| | | Tibial (AH) | | | Peroneal (TA) | | Median (APB) | | | Median (F2) | | Radial | | Sural | | Thenar | | Foot | | Palm | Sole |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CMAP | CV | F lat. | CMAP | CV | CMAP | CV | F lat. | SNAP | CV | SNAP | CV | SNAP | CV | CDT | WDT | CDT | WDT | SSR | SSR |
| **IV.B** | 1995 | **0.1** | **27** | **abs.** | **abs**. | **abs** | **2.3** | **40** | **55** | **abs.** | | **abs.** | | **abs.** | | | | | | | |
| **IV.D** | 1998 | **1.6** | **33** | **65** | 6.1 | 47 | 4.7 | **47** | **34** | **6** | 52 | 25 | 47 | **abs.** | | 0.9 | 1.3 | **14.5** | **19.0** | | |
| **IV.D** | 2001 | **0.1** | **30** | **abs.** | | | 5.4 | **45** | **38** | **6** | **43** | | | **abs.** | | 1.2 | 1.5 | **16.2** | **25.6** | | |
| **IV.D** | 2009 | **abs.** | | | 4.0 | 63 | **2.9** | **46** | **36** | **2** | **41** | **5** | 51 | **abs.** | | 2.5 | 2.6 | **abs.** | **abs.** | | |
| **IV.F** | 2008 | **2.3** | **39** | 59 | 3.0 | | 7.7 | 54 | 30 | 8 | 50 | 22 | 63 | **2** | 54 | | | | | | |
| **IV.F** | 2009 | **2.2** | **33** | **62** | | | 8.9 | 50 | 29 | 10 | 52 | | | **abs.** | | **5.0** | 2.6 | **abs.** | **abs.** | | |
| **IV.A** | 1993 | | | | **2.0** | **37** | | | | | | **abs.** | | **abs.** | | | | | | | |
| **IV.A** | 1994 | | | | | | **abs.** | | | **abs.** | | | | | | | | | | | |
| **V.B** | 2001 | 11.5 | | 48 | | | 12.3 | 50 | 27 | 16* | 61* | 23 | 64 | | | | | | | | |
| **V.B** | 2011 | 14.7 | 49 | 53 | | | 8.9 | 50 | 29 | 13 | 52 | | | 30 | 50 | 3.8 | 1.7 | **8.9** | **14.5** | 1.15 | **abs.** |
| **V.B** | 2012 | 11.8 | 47 | 53 | | | | | | | | | | 27 | 52 | 1.9 | 1.0 | **22.1** | **15.8** | 0.75 | **abs.** |

*Abnormal results in bold (reference values - National Hospital for Neurology, London).

Note that some values are omitted from the table where findings measurements were restricted to one investigation and individual.

abs. – absent

AH – abductor hallucis muscle

APB – abductor pollicis muscle

CDT - cold detection threshold, deviation form baseline (° C, pre 2008 variable, post 2008 32.0 ° C ).

CMAP – amplitude of the compound muscle action potential following distal stimulation (mV)

CV – conduction velocity (m/s)

F lat. – Minimal F wave latency (ms)

F2 – index finger. * Middle finger

SNAP – amplitude of the sensory nerve action potential (μV)

SSR – sympathetic skin response (mV)

TA – tibialis anterior muscle

WDT – warm detection threshold, deviation form baseline (° C)

**Table S3**

**Prion protein deposition in the peripheral nervous system and organs of case IV.F**

| Organ/tissue | Pattern of immunohistochemical staining for prion protein (ICSM35) | Extent of staining |
|---|---|---|
| **Dorsal root** | Granular staining with thin concentric staining around myelinated fibres seen in cross section. | ++ |
| **Dorsal root ganglion** | Circumferential staining around the majority of ganglion cells. | ++ |
| **Median and ulnar nerves** | Granular staining with thin concentric staining around myelinated fibres seen in cross section. | ++ |
| **Phrenic nerve** | Granular staining with thin concentric staining around myelinated fibres seen in cross section. | ++ |
| **Sympathetic nerve** | Granular staining with thin concentric staining around myelinated fibres seen in cross section. | ++ |
| **Skeletal muscle** | Fine punctate sarcoplasmic staining in a small proportion of fibres. | + |
| **Smooth muscle** | Coarse punctate staining. | ++ |
| **Lymph nodes (mediastinal and mesenteric)** | Minimal granular deposits in the stroma, secondary follicles not affected. Granular deposits more abundant in the capsule. | + |
| **Heart** | Widespread granular deposition between myocytes. | ++ |
| **Aorta** | Negative. | 0 |
| **Arteries** | Variably affected showing granular staining of the smooth muscle. | 0 - ++ |
| **Veins** | Variably affected by circumferential or patchy mural deposits. | 0 - ++ |
| **Lung** | Coarse granular staining in alveolar walls. | + - ++ |
| **Kidney** | Patchy distribution around tubules in the cortex and in the capsule, medulla negative. | ++ |
| **Liver** | Punctate deposition restricted to portal tract connective tissue. | + |
| **Pancreas** | Labeling of nerves and connective tissue around ducts. Parenchyma negative. | + |
| **Gallbladder** | Punctate staining of the mucosa and wall. Mucosa poorly preserved. | ++ |
| **Bladder** | Punctate staining of the mucosa and muscle. | ++ |
| **Adrenal cortex** | Widespread dot-like staining of the parenchyma. | ++ |
| **Small intestine** | Deposition in smooth muscle (mucosa poorly preserved). | ++ |
| **Colon** | Punctate staining of the lamina propria and muscularis mucosae with dot-like staining at the periphery of lymphoid aggregates but not involving follicular germinal centres. | ++ |
| **Uterus** | Strong positivity in smooth muscle and in the mucosa between glands. | ++ |
| **Skin** | Fine punctate staining in the upper dermis and basal layer of the epidermis with occasional fine threads in the subcutis. Punctate or continuous stain around eccrine sweat glands. Vessels and nerves stained as elsewhere. | + |

The findings for patient IV.D were highly similar. Semi-quantitative analysis of the extent of staining using a 4 point scale: 0 = no staining; + = minimal staining; ++ = moderate amount

of staining; +++ = extensive staining. Note: rectal mucosa and tonsil samples were not available for examination.

**Table S4**

**Semi-quantitative regional distribution of PrP and tau pathology in patient IV.A**

| Anatomical region | Prion protein deposition | | | Tau pathology | | |
|---|---|---|---|---|---|---|
| | Plaques | Vascular | Microglia | NFTs | NTs | ANs |
| Anterior frontal cortex | +++ | + | +++ | ++ | +++ | ++ |
| Motor cortex | +++ | + | +++ | + | +++ | + |
| Temporal cortex | +++ | ++ | +++ | ++ | +++ | ++ |
| Parietal cortex | +++ | + | +++ | + | ++ | + |
| Cingulate cortex | +++ | + | +++ | +++ | +++ | ++ |
| Occipital cortex | +++ | + | +++ | - | + | - |
| Dentate fascia | ++ | + | - | ++ | +++ | - |
| CA1 | + | ++ | ++ | + | +++ | + |
| Subiculum | +++ | + | ++ | ++ | +++ | ++ |
| Entorhinal cortex | +++ | + | +++ | +++ | +++ | + |
| Fusiform gyrus | +++ | + | ++ | +++ | +++ | + |
| Amygdala | ++ | + | ++ | +++ | +++ | ++ |
| Caudate | +++ | ++ | +++ | + | +++ | + |
| Putamen | +++ | + | ++ | + | +++ | - |
| Globus pallidus | + | +++ | ++ | - | +++ | + |
| Thalamus (AVTN) | ++ | ++ | + | + | +++ | - |
| Substantia nigra | + | +++ | + | ++ | +++ | - |
| Locus coeruleus | ++ | ++ | + | ++ | +++ | - |
| Medial lemniscus | - | + | - | N/A | ++ | N/A |
| Pontine nuclei | - | +++ | - | - | - | - |
| Cerebellar dentate | - | ++ | + | - | - | - |
| Cerebellar molecular layer | ++ | +++ | + | - | - | - |
| Cerebellar granular layer | + | ++ | - | - | - | - |
| Spinal cord grey matter | ++ | ++ | - | - | ++ | - |
| Spinal cord white matter | + | - | - | N/A | - | N/A |

Patients IV.D and IV.F showed a highly similar pattern and severity of pathology. AVTN = anteroventral thalamic nucleus, neurofibrillary tangle pathology (NFTs), neuropil threads (NTs) and abnormal neurites (ANs), N/A not applicable.

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